


## Article

# Foliar Mineral Treatments for The Reduction of Melon (*Cucumis melo* L.) Fruit Cracking

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**Abstract:** Fruit cracking affects many types of crops and is a major problem since the breakage of the surface of the fruit produces high economic losses. Numerous studies have looked at different ways to prevent this, mainly in melon, but with a low success rate. In this work, a standardisation of the induction of cracking is proposed that involves changes in the irrigation pattern (high conductivity or double irrigation). The prevention of the appearance of cracking was carried out through different foliar mineral treatments. The incidence of cracking was studied in relation to gas exchange variables and the concentrations of minerals in tissues. Our results show a more pronounced increase in cracking with double irrigation. Multiple elements were found to be associated with cracking such as B, Ca, K, Mg, Mn, Na, P, and Zn. Furthermore, foliar application of different microelements (B, Cu, Fe, Mn, Mo, and Zn) decreased the melon cracking incidence, thus assigning to the appropriate combination of these elements a crucial role in cracking amelioration.

**Keywords:** *Cucumis melo* L.; cracking; splitting; cracking induction; foliar nutrition; mineral content

## 1. Introduction

Cracking or splitting of fruit is a physical process that involves a mechanical break in the fruit cover due to an imbalance between the internal tension of the flesh and the tension of the external tissue of the cover [1]. Numerous causes can be distinguished, related to the climate (rainfall, temperature, air humidity, and solar radiation), soil conditions (fertility and physical, chemical and biological properties), fertilisers (components, bacteria, and chemicals), and crop management (soil-water-fertiliser relationship, pruning, and biological control) [2–5].

In addition to these external components, there are a series of internal variables that are of great importance with regard to the development of this condition, among which are the mineral composition of the plant [6,7] and the water content [8,9], since it is usually caused by an entry of water more rapid than the response of the plant [10]. In this regard, cracking has been related to certain genetic factors [11–13].

*Cucumis melo* L. (melon), which is a member of the Cucurbitaceae, is a species of high economic value, with the world production of melons in 2017 being nearly 32 Mt (<http://faostat.fao.org>). For melon, it has been described that the main causes of cracking are sudden changes in temperature between day and night, exposure to the sun during ripening, reduced spacing between plants, or high levels of humidity [10] with water playing a fundamental role. However, the induction of cracking and the interaction of all these factors with water are still poorly understood.

The development of cracking reduces the commercial value of melon fruit, and cracks are also a site of entry for infections by fungi and insects, leading to increased resource use and economic losses [3].

Numerous treatments have been tested, among which the application of foliar treatments can be highlighted. In some previous studies, it was attempted to prevent cracking through foliar treatments that restore the strength of the epidermis of the fruits and make them externally waterproof through kaolin-based particle film [14]. In others, hormone treatments ameliorated the incidence of cracking. Gibberellic acid (GA) and abscisic acid (ABA) improved cracking resistance through the upregulation of genes related to cuticle formation such as those encoding wax synthase or expansin-1 [9,15]. However, most of the studies on the prevention of cracking have been based on foliar treatments with mineral elements. Furthermore, it has been described that, due to the characteristics of the melon cuticle, the inclusion of a surfactant in the foliar treatments improved the mineral element adsorption by melon plants [16].

Currently, one of the foliar treatments used most to reduce cracking is calcium (Ca) application. Its effectiveness has been demonstrated in different types of crops such as *Litchi chinensis* Sonn. (litchi) [17], *Punica granatum* L. (pomegranate) [18], *Citrus limon* L. (lemon) [19], *Vaccinium corymbosum* L. (blueberry) [20], or *Prunus avium* L. (sweet cherry) [21]. It delays the ripening process by decreasing the respiration rate and ethylene production [22,23]. In addition to decreasing the incidence of cracking in other crops, Ca has been shown to improve the properties of both the melon fruit by increasing the diameter, skin thickness and firmness, and the plant, as well as increasing the plant weight, number of leaves, relative water content, and number of melons per plant [24].

In addition to Ca, many other elements have been associated with improvement of the cracking resistance and firmness of fruits, the most important being boron (B), magnesium (Mg), potassium (K), and zinc (Zn). These are mainly associated with improvement of the cell membrane, cell walls, or cuticles, through modification of the expression of genes or a merely structural function [17,19,25,26].

In this article, we study whether foliar application of three different treatments—Ca alone, Ca plus microelements of a commercial solution (B, molybdenum (Mo), and Zn), and another commercial solution of micro-elements (B, copper (Cu), iron (Fe), Mg, manganese (Mn), Mo, and Zn)—reduced the appearance of cracking in melon fruit. We compared the cracked part with the non-cracked part in the same fruit and also with the non-cracked fruits. The relationship between the gas exchange, mineral content in the leaves, and weight of fruit for the different irrigation and foliar treatments was determined.

## 2. Material and Methods

### 2.1. Experimental Conditions and Foliar Treatments

Experiments were conducted in the 2019 growing seasons in the fields of the company Sakata Seeds Iberica S.L.U., located in La Puebla (37°42′21.8″ N; 0°54′42.9″ W, Cartagena, Region of Murcia, Spain)—a semi-arid Mediterranean site with stable high temperatures and low humidity during the summer (May–September). The seedlings of *C. melo* var. Grand Riado were transplanted on 22-5-2019 and the fruit harvest was carried out two months later. The average of temperatures of the zone during the two months was around 25°.

Two weeks before the fruit harvest, three foliar treatments were applied with 200 mL per plant. The Ca treatment (Ca) consisted of 10 mM Ca (8 mM CaCl<sub>2</sub> and 2 mM CaSO<sub>4</sub>), the Ca and micronutrients treatment (Ca+m) consisted of Ca (13.36 mM), B (7.4 mM), Zn (2.29 mM), and Mo (0.02 mM) as a commercial mixture (Antisal gold, Nufol®), and the micronutrients treatment (m) was another commercial mixture, composed of Fe (0.9 mM), B (0.74 mM), Mn (0.53 mM), Cu (0.04 mM), Zn (0.14 mM), and Mo (0.004 mM) (Microfold, Nufol®). Deionised water was used as the control foliar treatment.

All plants were drip-irrigated by fertirrigation with KNO<sub>3</sub> (5.2 g m<sup>-2</sup>). Four days previous to fruit harvesting, the irrigation patterns were modified to induce cracking in the melons. Three groups of irrigation composed for three lines were set up: control (where the fertirrigation was maintained with no alteration), conductivity irrigation (the addition of 60 mM NaCl to the fertirrigation solution, to give

a conductivity of  $5.3 \text{ dS m}^{-1}$ ), and a double irrigation treatment (consisting of control fertirrigation followed by irrigation without nutrients but with the same volume of water).

The experimental design was completed randomized design (CRD). The four foliar treatments were distributed randomly in nine different lines. The lines were irrigated differently with the three irrigation treatment (three lines per irrigation treatment). Leaves, cracked, and non-cracked melons of each plot (36 in total) were recollected for the following analysis.

## 2.2. Fresh Weight and Incidence of Cracking

Two months after planting, all the melons of a commercial size (>two kg) were collected and weighed for each of the treatments to determine if the treatments affected negatively the correct development of the fruits. In addition, the fruits that presented cracking were registered two days before changing the irrigation treatments at harvest.

## 2.3. Physiological Determinations

The main physiological variables—transpiration rate ( $\text{mmol m}^{-2} \text{ s}^{-1}$ ), stomatal conductance ( $\text{mmol m}^{-2} \text{ s}^{-1}$ ), internal concentration of  $\text{CO}_2$  ( $\text{mmol m}^{-2} \text{ s}^{-1}$ ), and net photosynthetic rate ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )—were measured after the foliar treatments and changes in irrigation two days before the fruit harvest, on the second, third, and fourth fully-expanded leaves, 1 h after sunrise, using the TPS-2 Portable Photosynthesis System (PP Systems, Inc., Amesbury, MA, USA).

## 2.4. Analysis of Mineral Elements

We focused on those elements that were used in the foliar treatments for the analysis of the leaves, and on those elements that showed significant differences in the pulp and rind. The concentrations of B, Ca, K, Mg, Mn, Na, P, and Zn were analysed in the pulp and rind of non-cracked (nc) fruits and cracked fruits (for both the non-cracked (c-nc) area and the cracked (c) area).

The part of the fruit within 2 cm of the crack, in all directions, was collected and was considered as the cracked area. To separate the pulp from the rind, the first 3 cm were selected as the rind. The rest (the following 4–5 cm) was designated as the pulp, discarding the innermost area of the melon. All samples were dried and then ground finely in a mill grinder (model A10, IKA; Staufen, Germany). The samples were digested in a microwave oven (CEM Mars Xpress, Matthews, NC, USA) by  $\text{HNO}_3\text{--HClO}_4$  (2:1) acid digestion. The analysis of the elements was carried out using a Perkin–Elmer (Waltham, MA, USA) 5500 model ICP emission spectrophotometer (Iris Intrepid II, Thermo Electron Corporation, Franklin, TN, USA), at 589 nm, and their concentrations were expressed per g dry weight.

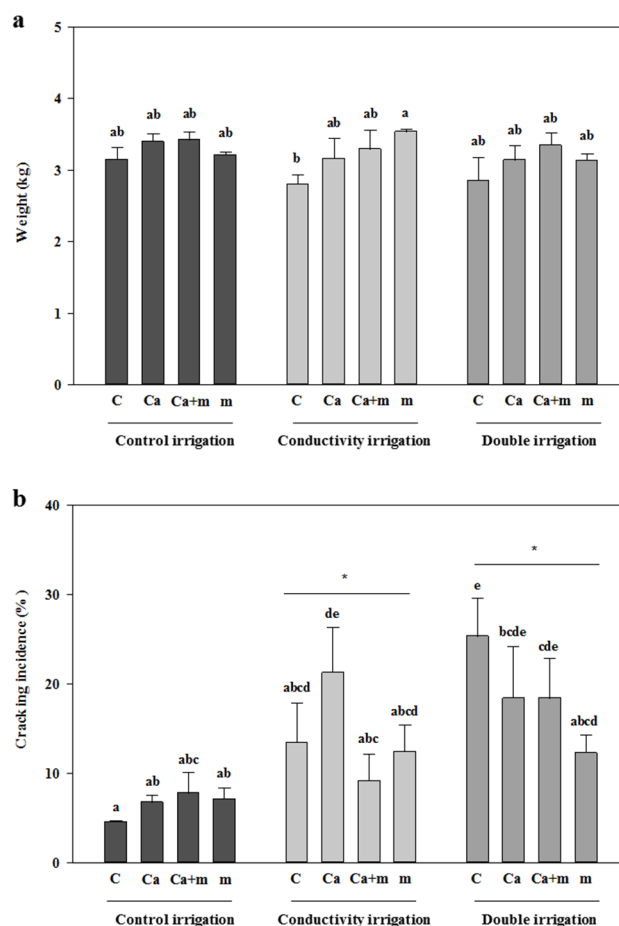
## 2.5. Data Analysis

Statistical analyses were performed using the SPSS 25.0.0.1 software package. All data were analysed using one-way ANOVA, followed by Duncan's multiple comparison test. Significant differences between the values of each determination were determined at  $p \leq 0.05$ , according to Duncan's test. The values presented are the means  $\pm$  SE. The correlation matrix generated by the Pearson coefficient correlation and principal components analysis was performed for the pulp and rind tissues with all elements applied in foliar treatments and the analysis results of the mineral nutrients. The Kaiser-Meyer-Olkin (KMO) measure of sampling adequacy was, in both cases, higher than 0.5 (0.809 for pulp and 0.773 for rind), and the Bartlett's test of sphericity value was, in both cases, lower than 0.05 (0.00 for pulp and rind), indicating that a factor analysis was useful with our data. The conventional approach to interpreting a correlation coefficient correlation selected were: very strong correlation (greater than 0.900), strong correlation (between 0.700 and 0.899), moderate correlation (between 0.400 and 0.699), and weak correlation (between 0 and 0.399) [27].

### 3. Results

#### 3.1. Fresh Weight and Incidence of Cracking

The weight of the melons (Figure 1a) remained very homogeneous across the distinct irrigation and foliar treatments, except for high conductivity irrigation, for which there were significant increases in plants receiving foliar treatment m, compared to its control.



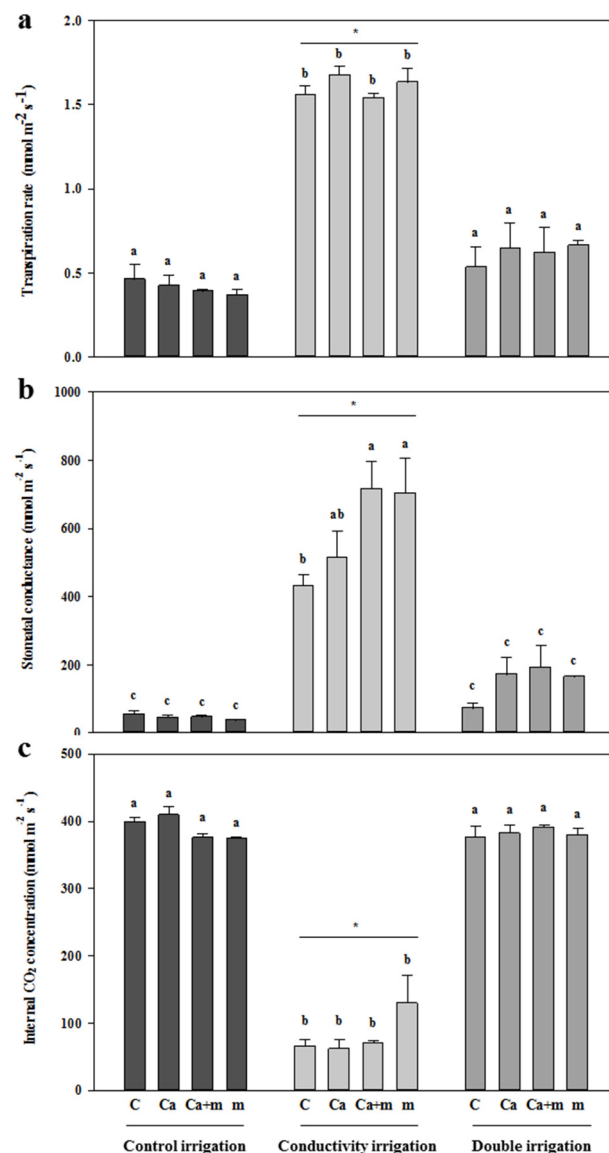
**Figure 1.** (a) Fresh fruit weight of all commercial-size melons (more than 2 kg). (b) Percentage cracking of commercial-size melons (more than 2 kg). Treatment Ca consisted of 8 mM  $\text{CaCl}_2$  and 2 mM  $\text{CaSO}_4$ , treatment Ca+m consisted of a commercial mixture (Antisal gold, Nufol®), and treatment m another commercial mixture (Microfold, Nufol®). Statistical analysis was performed using SPSS 25.0.0.1. The values are the means  $\pm$  SE of 13–20 individual analyses. Columns with different letters differ significantly according to Duncan's test ( $p = 0.05$ ). Significant differences between irrigation treatments are marked with \*.

Regarding the cracking percentage (Figure 1b), both irrigation treatments induced cracking, significantly increasing the incidence relative to the control. Furthermore, the cracking achieved with double irrigation was also significantly higher than that achieved with conductivity irrigation. Indeed, the highest value occurred in the control foliar treatment of the double irrigation treatment, followed by the Ca foliar treatment with conductivity irrigation, both being significantly higher than the respective foliar treatments under control irrigation. The m foliar treatment significantly reduced the incidence of cracking caused by double irrigation.

The total production of melons was similar in all applied foliar treatments without significant differences between them (data not shown).

### 3.2. Physiological Determinations

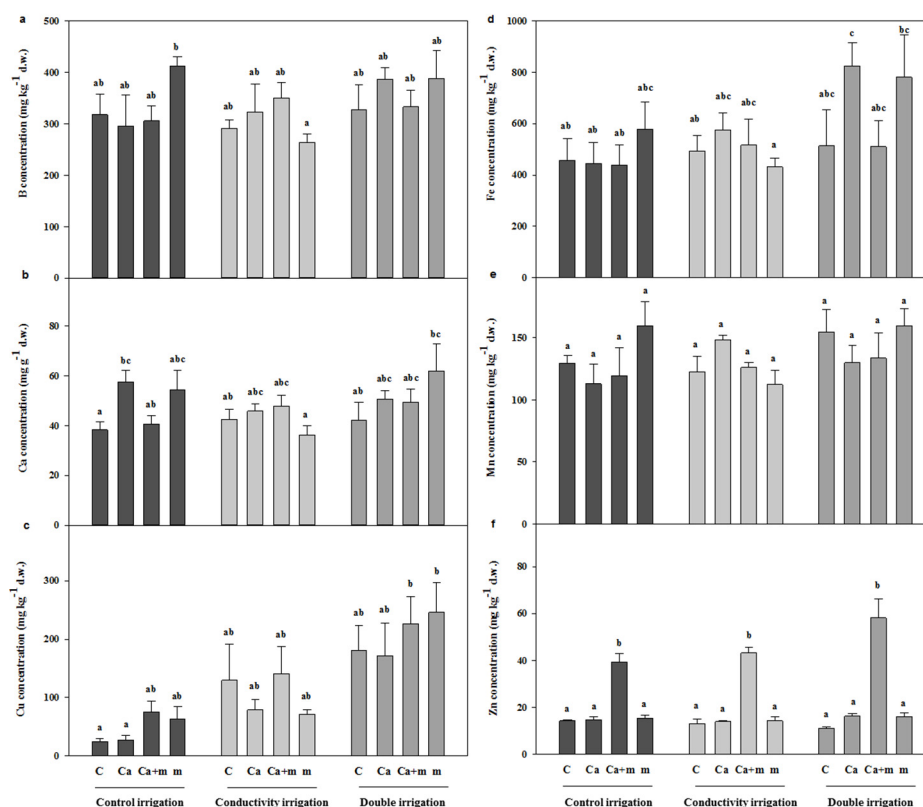
Transpiration (Figure 2a) and stomatal conductance (Figure 2b) followed the same pattern: the control and double irrigation treatment had similar values, without significant differences between them, whereas the conductivity irrigation treatment significantly increased the values of both variables. Additionally, the stomatal conductance increased significantly with the foliar Ca+m and m treatments under conductivity irrigation. The internal CO<sub>2</sub> concentration (Figure 2c) showed the opposite pattern with respect to the previously mentioned physiological variables: the control and the double irrigation gave higher values than the conductivity irrigation. No significant differences were found between the foliar treatments.



**Figure 2.** The main physiological determinations, measured after two months of growth: (a) transpiration rate (mmol m<sup>-2</sup> s<sup>-1</sup>), (b) stomatal conductance (mmol m<sup>-2</sup> s<sup>-1</sup>), and (c) internal concentration of CO<sub>2</sub> (mmol m<sup>-2</sup> s<sup>-1</sup>). Statistical analysis was performed using SPSS 25.0.0.1. The values are the means ± SE of three independent measurements on three adult leaves per plant for three plants per treatment ( $n = 9$ ). Columns with different letters differ significantly according to Duncan's test ( $p = 0.05$ ). Significant differences between irrigation treatments are marked with \*.

### 3.3. Analysis of Mineral Elements in Leaves

In general, the foliar treatments, independently of the type of irrigation, did not significantly modify the concentrations of B (Figure 3a), Cu (Figure 3c), Fe (Figure 3d), or Mn (Figure 3f) in leaves. However, significant differences were observed between the foliar treatments for Ca (Figure 3b) with an increase under control irrigation due to the Ca foliar treatment (with respect to its control). For Zn (Figure 3f), the Ca+m foliar treatment increases the Zn levels in all three irrigation treatments.



**Figure 3.** Leaf cation concentrations: (a) B (mg kg<sup>-1</sup>), (b) Ca (mg g<sup>-1</sup>), (c) Cu (mg kg<sup>-1</sup>), (d) Fe (mg kg<sup>-1</sup>), (e) Mn (mg kg<sup>-1</sup>), and (f) Zn (mg kg<sup>-1</sup>). The Mo levels were not detectable (data not shown). Statistical analysis was performed using SPSS 25.0.0.1. The values are the means  $\pm$  SE of three individual analyses. Columns with different letters differ significantly, according to Duncan's test ( $p = 0.05$ ).

On the other hand, the irrigation treatments produced significant differences for most of the nutrients except Mn (Figure 3e) and Zn (Figure 3f). For B (Figure 3a), in the m foliar treatment, we found differences between the control and conductivity irrigation, with a significant reduction in the latter. Regarding Ca (Figure 3b), we also found significant reductions, relative to the control, for the m treatment of the double irrigation and conductivity irrigation treatments. The Cu results (Figure 3c) show that the only differences were between the control and Ca foliar treatments with control irrigation and between the Ca+m and m foliar treatments with double irrigation. Regarding Fe (Figure 3d), we found significant differences among the control, Ca and Ca+m foliar treatments for the control irrigation, between the control and the m treatment for conductivity irrigation and between the control and the Ca treatment for double irrigation. In addition, for treatment m, significant differences in Fe were observed between conductivity irrigation and double irrigation, which is higher in the latter.

### 3.4. Analysis of Mineral Elements in Fruit

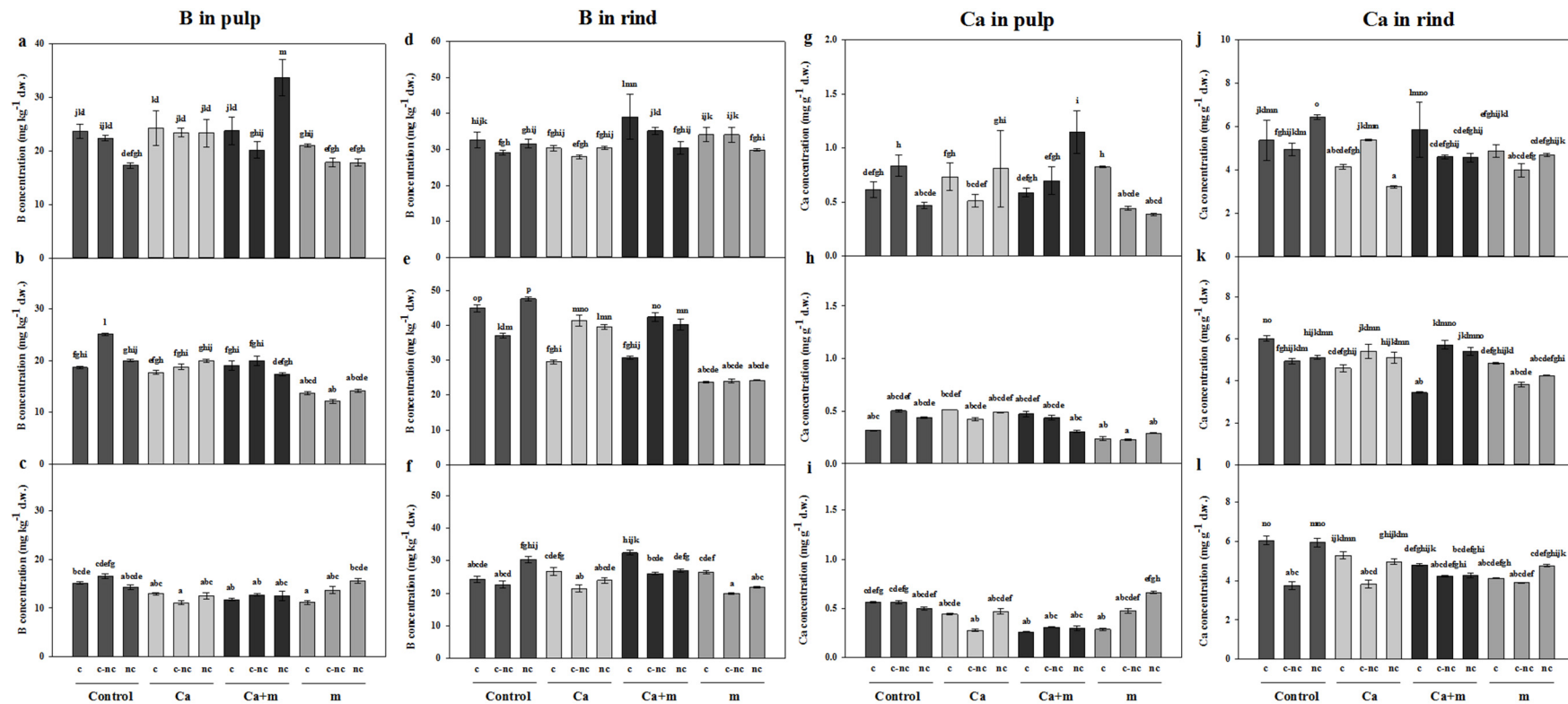
The mineral nutrients were analysed in both non-cracked and cracked fruits. Also, in cracked fruits, the non-cracked part was analysed separately from the cracked part. There were differences only

in some minerals, as illustrated in Figures 4–7. The results for the nutrients that showed differences between cracked (c) and non-cracked (nc and c-nc) fruits will be described hereafter.

The B concentration in pulp was generally higher with control irrigation (Figure 4a) than with conductivity irrigation (Figure 4b) and was much higher than with double irrigation (Figure 4c). With control irrigation (Figure 4a), a significant increase for c areas with respect to nc melons was found in the control foliar treatment and a significant decrease for c areas with respect to nc melons was found in the Ca+m foliar treatment. With conductivity irrigation (Figure 4b), a significant decrease in the B concentration in c areas with respect to c-nc areas occurred in the control foliar treatment. With double irrigation (Figure 4c), the B concentration in c areas was significantly lower than in nc melons in the m foliar treatment. The B concentration in rind for control irrigation (Figure 4d) was generally higher than of double irrigation (Figure 4e) and was similar to that of conductivity irrigation (Figure 4f). With control irrigation (Figure 4d), the B concentration was significantly increased in c areas with respect to nc melons in the Ca+m foliar treatment. With conductivity irrigation (Figure 4e), a significant increase in the B concentration in c areas with respect to c-nc areas in the control foliar treatment occurred and a significant decrease in c areas in Ca and Ca+m foliar treatment with respect to both nc and c-nc is shown. With double irrigation (Figure 4f), the B concentration in c areas was significantly lower than in nc melons in the control foliar treatment and there was an increase in c areas with respect to c-nc areas in the Ca foliar treatment, which is an increase in c areas with respect to c-nc areas and nc melons in the Ca+m foliar treatment and an increase in c areas with respect to c-nc areas in the m foliar treatment.

The Ca concentration in pulp was generally higher with control irrigation (Figure 4g) than with conductivity irrigation (Figure 4h) or double irrigation (Figure 4i). With control irrigation (Figure 4g), a significant decrease was found in c areas with respect to nc melons in the Ca+m treatment, together with a significant increase in c areas with respect to c-nc areas and nc melons in the m foliar treatment. With conductivity irrigation (Figure 4h), no significant differences were found between the c areas and the c-nc areas or nc fruits in any foliar treatment. With double irrigation (Figure 4i), the Ca concentration in c areas was significantly lower than in nc melons in the m foliar treatment. The Ca concentration in rind was similar among all irrigation treatments (Figure 4j–l). With control irrigation (Figure 4j), there was a significant decrease in the Ca concentration in c areas with respect to nc melons in the control foliar treatment, a significant decrease in c with respect to c-nc melons in the Ca foliar treatment, and an increase in c with respect to c-nc areas and nc melons in the Ca+m foliar treatment. With conductivity irrigation (Figure 4k), there was a significant increase in the Ca concentration in c areas with respect to c-nc areas in the control foliar treatment and a significant decrease in the Ca+m foliar treatment for both nc and c-nc. With double irrigation (Figure 4l), a significant increase in the Ca concentration in c areas with respect to c-nc areas in both the control and the Ca foliar treatment was observed.



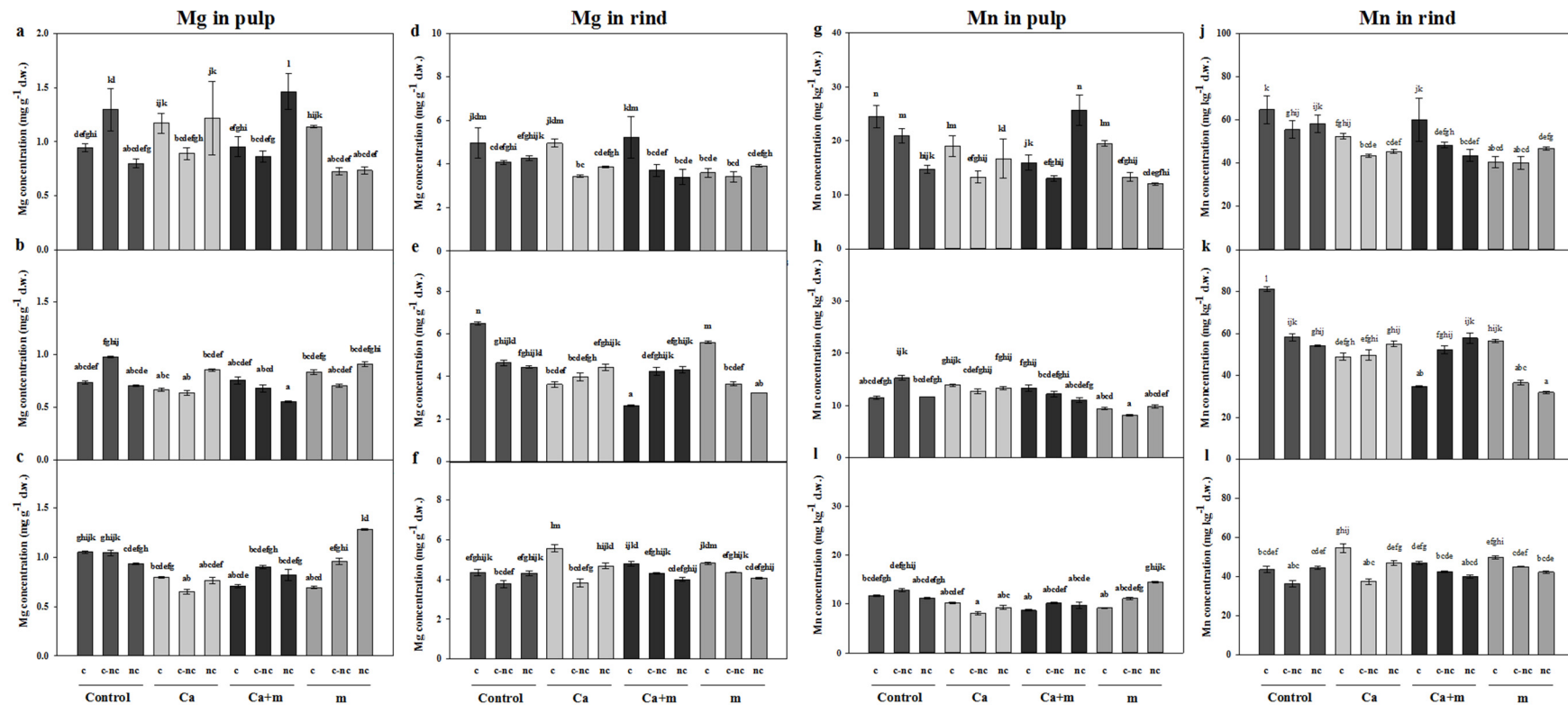


**Figure 4.** B concentration (dry weight basis) in pulp, (a) control irrigation, (b) conductivity irrigation, and (c) double irrigation, and rind, (d) control irrigation, (e) conductivity irrigation, and (f) double irrigation. Ca concentration (dry weight basis) in pulp, (g) control irrigation, (h) conductivity irrigation, and (i) double irrigation, and rind, (j) control irrigation, (k) conductivity irrigation, and (l) double irrigation. The Ca treatment consisted of 8 mM  $\text{CaCl}_2$  and 2 mM  $\text{CaSO}_4$ , the Ca+m treatment consisted of a commercial mixture (Antisal gold, Nufol®) and the m treatment consisted of another commercial mixture (Microfold, Nufol®). Codes: c, cracked region in cracked fruit, c-nc, non-cracked region in cracked fruit, nc, non-cracked fruit. Statistical analysis was performed using SPSS 25.0.0.1. and included all the irrigation treatments, separating each element and each type of tissue. The values are the means  $\pm$  SE of three individual analyses. Columns with different letters differ significantly according to Duncan's test ( $p = 0.05$ ).



With control irrigation, the Mg concentration in pulp (Figure 5a) was generally higher than with double irrigation (Figure 5c) and much higher than with conductivity irrigation (Figure 5b). With control irrigation (Figure 5a), there was a significant decrease in the Mg concentration in c areas with respect to c-nc areas in the control foliar treatment, a significant increase in c areas with respect to c-nc areas in the Ca foliar treatment, a significant decrease in c areas with respect to nc melons in the Ca+m foliar treatment, and a significant increase in c areas with respect to c-nc areas and nc melons in the m foliar treatment. For conductivity irrigation (Figure 5b), no significant differences were found between the c areas and the c-nc areas or nc fruits in any foliar treatment. For double irrigation (Figure 5c), the Mg concentration in c areas was significantly lower than in nc melons in the m foliar treatment. The Mg concentration in rind was similar among all irrigation treatments (Figure 5d–f). For control irrigation (Figure 5d), there was a significant increase in the Mg concentration in c areas with respect to c-nc areas in the control foliar treatment, and a significant increase in c areas with respect to c-nc areas and nc melons in the Ca and Ca+m foliar treatments. For conductivity irrigation (Figure 5e), the Mg concentration in c areas showed a significant increase with respect to c-nc areas and nc melons in the control and m foliar treatments, and a significant decrease with respect to both nc melons and c-nc areas in the Ca+m foliar treatment. With double irrigation (Figure 5f), a significant increase in the Mg concentration in c areas with respect to c-nc areas in the Ca foliar treatment, can be seen.

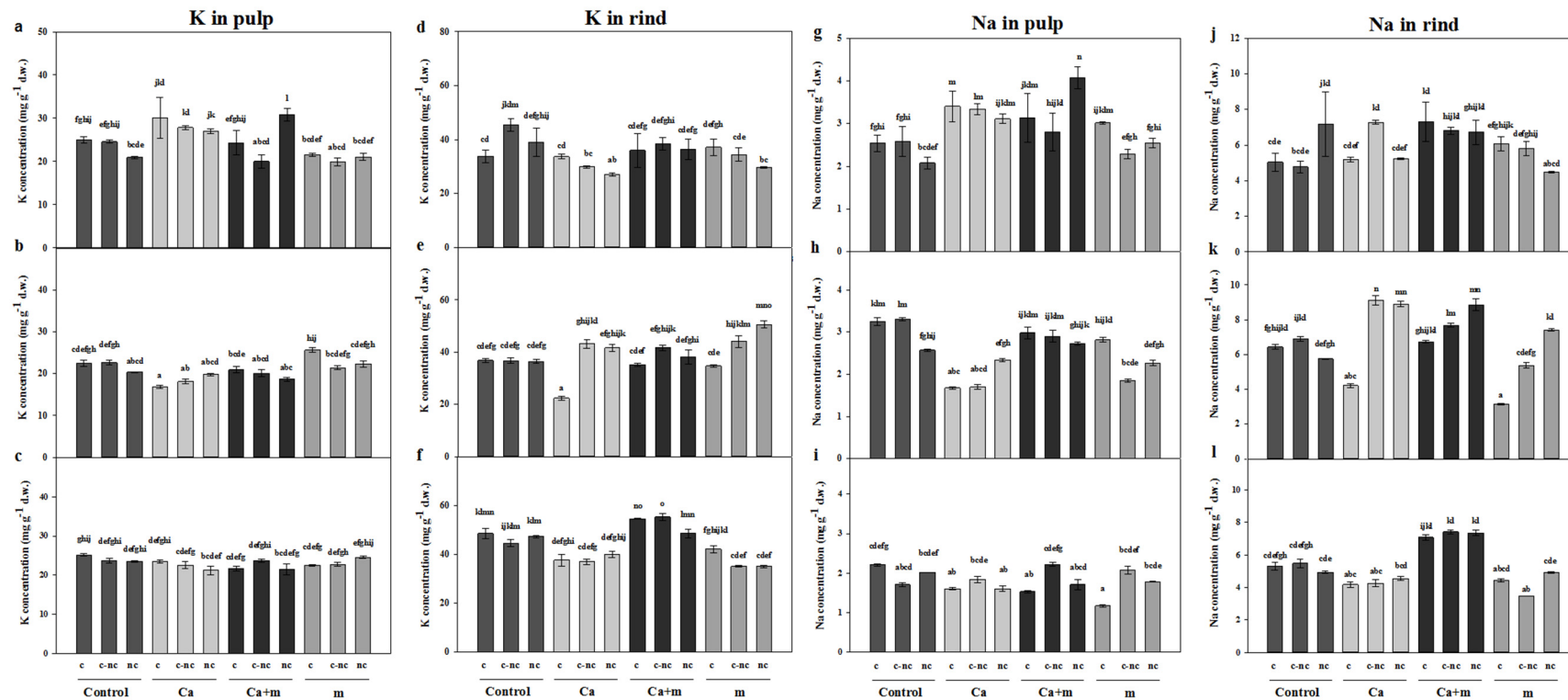
The Mn concentration in pulp was generally higher for control irrigation (Figure 5g) than for conductivity (Figure 5h) or double irrigation (Figure 5i). For control irrigation (Figure 5g), there was a significant increase in the Mn concentration in c areas with respect to c-nc areas and nc melons in the control and m foliar treatments and in c areas with respect to c-nc areas in the Ca foliar treatment as well as a significant decrease in c areas with respect to nc melons in the Ca+m foliar treatment. With conductivity irrigation (Figure 5h), the only significant effect was a decrease in c with respect to c-nc areas in the control foliar treatment. With double irrigation (Figure 5i), the Mn concentration was significantly lower in c areas than in nc melons in the m foliar treatment. The Mn concentration in rind was similar in the control and conductivity irrigation treatments (Figure 5j,k) but lower with double irrigation (Figure 5l). For control irrigation (Figure 5j), a significant increase in the Mn concentration in c with respect to c-nc areas in the control and Ca foliar treatments can be observed as well as a significant increase in c areas with respect to c-nc areas and nc melons in the Ca+m foliar treatment. For conductivity irrigation (Figure 5k), a significant increase in the Mn concentration in c areas with respect to c-nc areas and nc melons in the control and m foliar treatments, and a significant decrease with respect to both nc melons and c-nc areas in the Ca+m foliar treatment, can be seen. With double irrigation (Figure 5l), a significant increase in the Mn concentration was found in c with respect to c-nc areas in the Ca foliar treatment.



**Figure 5.** Mg concentration (dry weight basis) in pulp, (a) control irrigation, (b) conductivity irrigation, and (c) double irrigation, and rind, (d) control irrigation, (e) conductivity irrigation, and (f) double irrigation. Mn concentration (dry weight basis) in pulp, (g) control irrigation, (h) conductivity irrigation, and (i) double irrigation, and rind, (j) control irrigation, (k) conductivity irrigation, and (l) double irrigation. The Ca treatment consisted of 8 mM CaCl<sub>2</sub> and 2 mM CaSO<sub>4</sub>, the Ca+m treatment consisted of a commercial mixture (Antisal gold, Nufol®) and the m treatment consisted of another commercial mixture (Microfold, Nufol®). Codes: c, cracked region in cracked fruit. c-nc, non-cracked region in cracked fruit. nc, non-cracked fruit. Statistical analysis was performed using SPSS 25.0.0.1. and included all the irrigation treatments, separating each element and each type of tissue. The values are the means  $\pm$  Standard Error of three individual analyses. Columns with different letters differ significantly according to Duncan's test ( $p = 0.05$ ).

The K concentration in pulp was generally similar with the control (Figure 6a) and double irrigation (Figure 6c), but was lower with conductivity irrigation (Figure 6b). For control irrigation (Figure 6a), a significant increase in the K concentration in c areas with respect to nc melons was found in the control foliar treatment while a significant increase in c areas with respect to c-nc areas and a decrease with respect to nc melons were found in the Ca+m foliar treatment. For conductivity irrigation (Figure 6b), the only significant difference was the higher value in c with respect to c-nc areas in the m foliar treatment. For double irrigation (Figure 6c), no significant difference was observed in any foliar treatment. The K concentration in rind was similar in all irrigation treatments (Figure 6d–f). For control irrigation (Figure 6d), there was a significant decrease in the K concentration in c with respect to c-nc areas in the control foliar treatment, and a significant increase in c areas with respect to nc melons in the Ca and m foliar treatments. For conductivity irrigation (Figure 6e), the K concentration in c areas was significantly lower than in c-nc areas and nc melons in the Ca and m foliar treatments. With double irrigation (Figure 6f), no significant differences were observed within the foliar treatments, among the cracked and non-cracked zones or non-cracked melons.

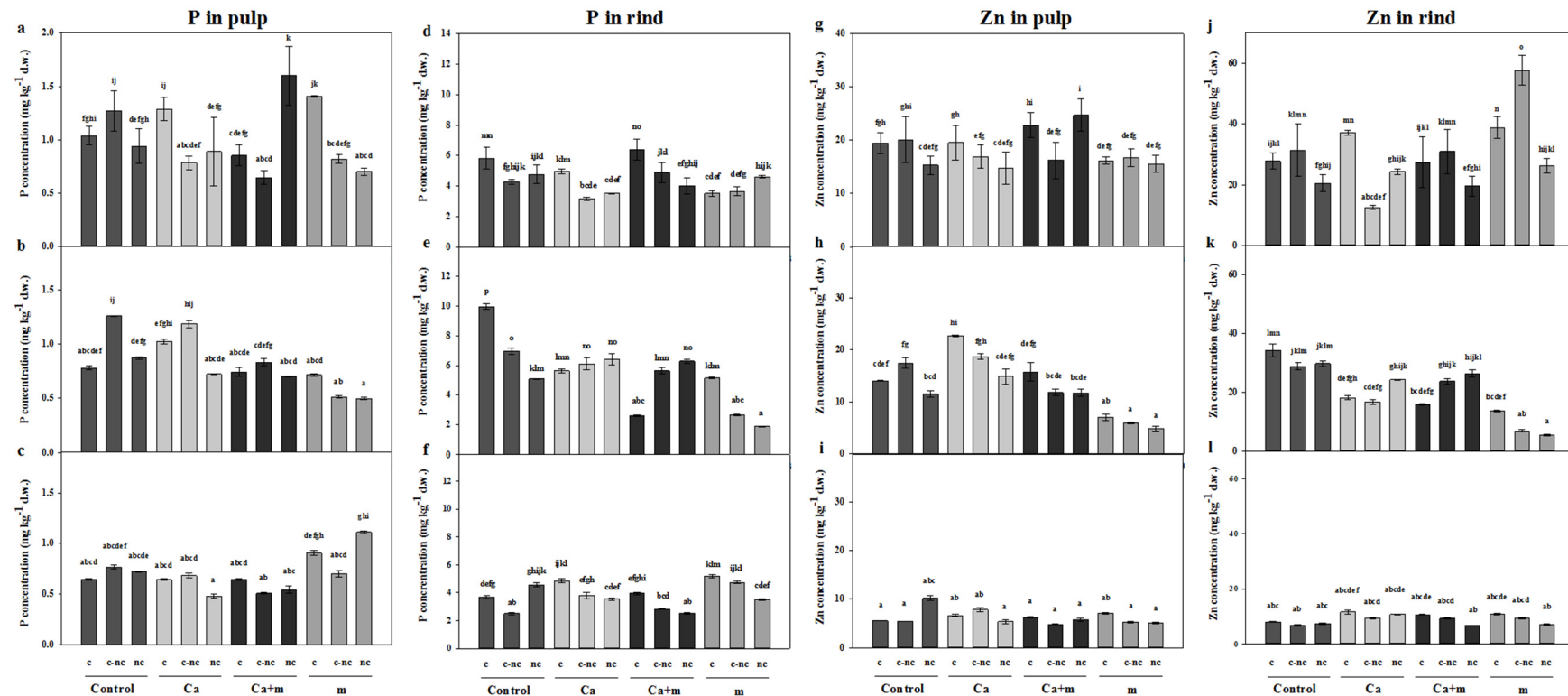
The Na concentration in pulp was generally similar for control and conductivity irrigation (Figure 6g,h), and lower for double irrigation (Figure 6i). With control irrigation (Figure 6g), a significant decrease in the Na concentration in c areas with respect to nc melons in the Ca+m foliar treatment, and a significant increase in c with respect to c-nc areas in the m foliar treatment were observed. For conductivity irrigation (Figure 6h), an increase in the Na concentration in c areas with respect to nc melons in the control foliar treatment can be seen as well as a decrease in c areas with respect to nc melons in the Ca foliar treatment and a significant increase in c with respect to c-nc areas in the m foliar treatment. For double irrigation (Figure 6i), there was a significant decrease in the Na concentration in c areas with respect to c-nc areas in the Ca+m foliar treatment and a decrease in c areas with respect to c-nc areas and nc melons in the m foliar treatment. The Na concentration in rind was similar in the control and double irrigation treatments (Figure 6j,l) and higher with conductivity irrigation (Figure 6k). With control irrigation (Figure 6j), there was a significant decrease in the Na concentration in c areas with respect to nc melons in the control foliar treatment, which is a significant decrease in c areas with respect to c-nc areas in the Ca foliar treatment and a significant increase in c areas with respect to nc melons in the m foliar treatment. With conductivity irrigation (Figure 6k), a significant decrease in the Na concentration in c areas with respect to c-nc areas and nc melons in the Ca and m foliar treatments, and a significant decrease with respect to nc melons in the Ca+m foliar treatment occurred. With double irrigation (Figure 6l), significant differences were not observed.



**Figure 6.** The K concentration (dry weight basis) in pulp, (a) control irrigation, (b) conductivity irrigation, and (c) double irrigation, and rind, (d) control irrigation, (e) conductivity irrigation, and (f) double irrigation. The Na concentration (dry weight basis) in pulp, (g) control irrigation, (h) conductivity irrigation, and (i) double irrigation, and rind, (j) control irrigation, (k) conductivity irrigation, and (l) double irrigation. The Ca treatment consisted of 8 mM  $\text{CaCl}_2$  and 2 mM  $\text{CaSO}_4$ , the Ca+m treatment consisted of a commercial mixture (Antisal gold, Nufol®) and the m treatment consisted of another commercial mixture (Microfold, Nufol®). Codes: c, cracked region in cracked fruit. c-nc, non-cracked region in cracked fruit. nc, non-cracked fruit. Statistical analysis was performed using SPSS 25.0.0.1 and included all the irrigation treatments, separating each element and each type of tissue. The values are the means  $\pm$  SE of three individual analyses. Columns with different letters differ significantly according to Duncan's test ( $p = 0.05$ ).

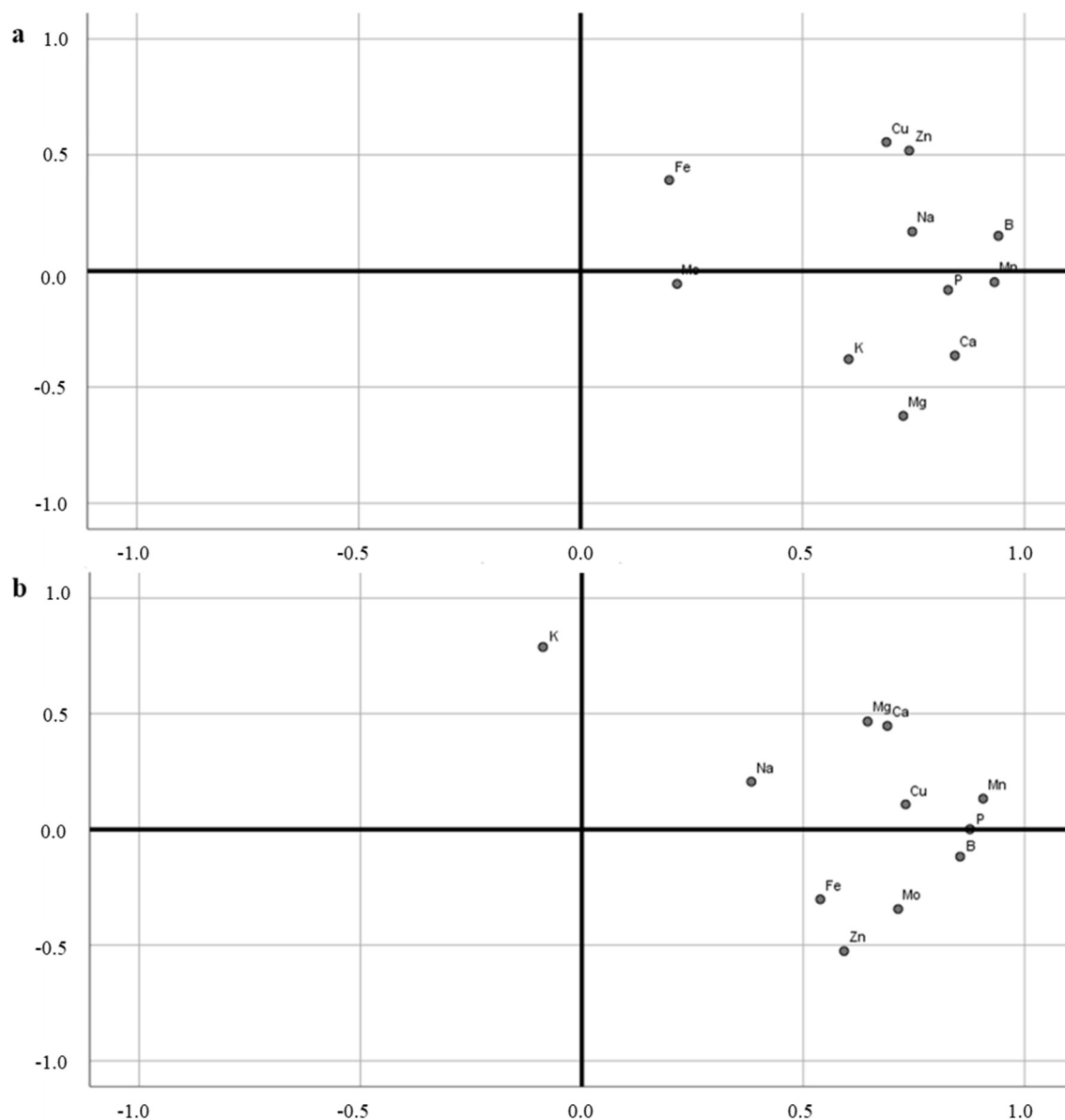
The P concentration in pulp for control irrigation (Figure 7a) was generally higher than for conductivity irrigation (Figure 7b) and much higher than for double irrigation (Figure 7c). With control irrigation (Figure 7a), a significant increase in the P concentration in c areas with respect to c-nc areas and nc melons was found in the Ca and m foliar treatments as well as a significant decrease in c areas with respect to nc melons in the Ca+m foliar treatment. With conductivity irrigation (Figure 7b), the only significant difference was a decrease in c with respect to c-nc areas in the control foliar treatment. With double irrigation (Figure 7c), the c areas did not exhibit significant differences in any foliar treatment among cracked areas and non-cracked areas or melons. The P concentration in rind was similar in the control and conductivity irrigation treatments (Figure 7d,e) and lower with double irrigation (Figure 7f). With control irrigation (Figure 7d), a significant increase in the P concentration in c areas with respect to c-nc areas and nc melons in the control, Ca and Ca+m foliar treatments, and a significant decrease in c areas with respect to nc melons in the m foliar treatment can be observed. With conductivity irrigation (Figure 7e), a significant increase in the P concentration in c areas with respect to c-nc areas and nc melons in the control and m foliar treatments as well as a significant decrease in c areas with respect to c-nc areas and nc melons in the Ca+m treatment occurred. With double irrigation (Figure 7f), there was a significant increase in the P concentration in c areas with respect to c-nc areas in the control foliar treatment, a significant increase in c areas with respect to c-nc areas, nc melons in the Ca and Ca+m foliar treatments, and a significant increase in c areas with respect to nc melons in the m foliar treatment.

The Zn concentration in pulp was generally higher in control irrigation (Figure 7g) than in conductivity irrigation (Figure 7h) and, particularly, double irrigation (Figure 7i). For control irrigation (Figure 7g), the Zn concentration in c areas was significantly higher than in c-nc areas in the Ca+m foliar treatment. With conductivity irrigation (Figure 7h), there was an increase in the Zn concentration in c areas with respect to nc melons in the Ca foliar treatment. With double irrigation (Figure 7i), no differences were observed. The Zn concentration in rind was generally higher with control irrigation (Figure 7j) than with conductivity irrigation (Figure 7k) and, especially, double irrigation (Figure 7l). For control irrigation (Figure 7j), there was a significant increase in the Zn concentration in c areas with respect to c-nc areas and nc melons in the Ca foliar treatment and, for the m foliar treatment, the c areas showed a significant decrease with respect to c-nc areas and an increase with respect to nc melons. For conductivity irrigation (Figure 7k), a significant decrease in the Zn concentration in c areas with respect to nc melons in the Ca+m foliar treatment, and a significant increase in c areas with respect to c-nc areas and nc melons in the m foliar treatment occurred. With double irrigation (Figure 7l), no significant differences were observed.



**Figure 7.** The P concentration (dry weight basis) in pulp, (a) control irrigation, (b) conductivity irrigation, and (c) double irrigation, and rind, (d) control irrigation, (e) conductivity irrigation, and (f) double irrigation. The Zn concentration (dry weight basis) in pulp, (g) control irrigation, (h) conductivity irrigation, and (i) double irrigation, and rind, (j) control irrigation, (k) conductivity irrigation, and (l) double irrigation. The Ca treatment consisted of 8 mM  $\text{CaCl}_2$  and 2 mM  $\text{CaSO}_4$ , the Ca+m treatment consisted of a commercial mixture (Antisal gold, Nufol®) (Table 1), and the m treatment consisted of another commercial mixture (Microfold, Nufol®). Codes: c, cracked region in cracked fruit. c-nc, non-cracked region in cracked fruit. nc, non-cracked fruit. Statistical analysis was performed using SPSS 25.0.0.1. and included all the irrigation treatments, separating each element and each type of tissue. The values are the means  $\pm$  SE of three individual analyses. Columns with different letters differ significantly according to Duncan's test ( $p = 0.05$ ).

The correlation matrix and the principal component analysis show that some elements are associated with each other, strengthening the trends that we detected with the previous analysis. In pulp (Table 1) (Figure 8a), B is strongly associated with Mn, but also with other elements such as Ca, Na, P, and Zn, Ca is closely associated with Mg and Mn, and also moderately correlated with B and P, while K is correlated with Mg. In addition, Mg correlated with Mn and P, while P correlated moderately with Zn. In the rind (Table 2) (Figure 8b), we see fewer strong relationships and only the ones of B and Mn with P. We see other weak correlations, such as B with Ca, Cu, Mg, Mn, Na, P, and Zn, Ca with Mg and Mn, Mg with P, Mn with Mo, Mo with P and Zn, P with S, and S with Zn. In the rind, K and Na did not correlate with any other element.



**Figure 8.** Principal component analysis (PCA) of pulp (a) and rind (b). Component graph in two-dimensional rotated space. Statistical analysis was performed using SPSS 25.0.0.1., including all variables in the analysis.



**Table 1.** Correlation matrix for elements in the rind. Statistical analysis was performed using SPSS 25.0.0.1. Correlations greater than 0.900 were considered very strong and are marked with \*\*\*. Correlations between 0.700 and 0.899 were considered strong correlation and are marked with \*\*. Correlations between 0.400 and 0.699 were considered moderate correlation and are marked with \*. Correlations between 0 and 0.399 were considered weak correlation and are not marked. The KMO test score was 0.828. Bartlett's test score was 0. Each element corresponds to independent measurements and 108 individual analyses.

	B	Ca	Cu	Fe	K	Mg	Mn	Mo	Na	P	Zn
B	1.000	0.749 *	0.676 *	0.175	0.474 *	0.556 *	0.849 **	0.198	0.799 *	0.735 *	0.775 *
Ca	0.749 *	1.000	0.299	0.059	0.472 *	0.832 **	0.843 **	0.140	0.477 *	0.752 *	0.495 *
Cu	0.676 *	0.299	1.000	0.246	0.376	0.162	0.603 *	0.074	0.541 *	0.508 *	0.756 *
Fe	0.175	0.059	0.246	1.000	0.074	0.036	0.179	−0.008	0.196	0.087	0.138
K	0.474 *	0.472 *	0.376	0.074	1.000	0.673 *	0.474 *	0.069	0.512 *	0.352	0.145
Mg	0.556 *	0.832 **	0.162	0.036	0.673 *	1.000	0.710 *	0.148	0.431 *	0.619 *	0.198
Mn	0.849 **	0.843 **	0.603 *	0.179	0.474 *	0.710 *	1.000	0.182	0.565 *	0.803 **	0.682 *
Mo	0.198	0.140	0.074	−0.008	0.069	0.148	0.182	1.000	0.138	0.170	0.145
Na	0.799 *	0.477 *	0.541 *	0.196	0.512 *	0.431 *	0.565 *	0.138	1.000	0.442 *	0.554 *
P	0.735 *	0.752 *	0.508 *	0.087	0.352	0.619 *	0.803 **	0.170	0.442 *	1.000	0.581 *
Zn	0.775 *	0.495 *	0.756 *	0.138	0.145	0.198	0.682 *	0.145	0.554 *	0.581 *	1.000

**Table 2.** Correlation matrix for elements in the rind. Statistical analysis was performed using SPSS 25.0.0.1. Correlations greater than 0.900 were considered very strong and are marked with \*\*\*. Correlations between 0.700 and 0.899 were considered strong correlation and are marked with \*\*. Correlations between 0.400 and 0.699 were considered moderate correlation and are marked with \*. Correlations between 0 and 0.399 were considered weak correlation and are not marked. The KMO test score was 0.753. Bartlett's test score was 0. Each element corresponds to independent measurements and 108 individual analyses.

	B	Ca	Cu	Fe	K	Mg	Mn	Mo	Na	P	Zn
B	1.000	0.510 *	0.600 *	0.423 *	−0.023	0.305	0.618 *	0.706 **	0.590 *	0.673 *	0.615 *
Ca	0.510 *	1.000	0.435 *	0.261	0.191	0.568 *	0.659 *	0.334	0.324	0.558 *	0.172
Cu	0.600 *	0.435 *	1.000	0.318	−0.037	0.500 *	0.643 *	0.362	0.226	0.602 *	0.332
Fe	0.423 *	0.261	0.318	1.000	−0.172	0.180	0.414	0.444 *	0.176	0.367	0.330
K	−0.023	0.191	−0.037	−0.172	1.000	0.131	−0.096	−0.173	0.408 *	−0.234	−0.293
Mg	0.305	0.568 *	0.500 *	0.180	0.131	1.000	0.809 **	0.176	−0.072	0.686 *	0.131
Mn	0.618 *	0.659 *	0.643 *	0.414 *	−0.096	0.809 **	1.000	0.524 *	0.139	0.881 **	0.450 *
Mo	0.706 *	0.334	0.362	0.444 *	−0.173	0.176	0.524 *	1.000	0.415 *	0.541 *	0.540 *
Na	0.590 *	0.324	0.226	0.176	0.408 *	−0.072	0.139	0.415 *	1.000	0.146	0.161
P	0.673 *	0.558 *	0.602 *	0.367	−0.234	0.686 *	0.881 **	0.541 *	0.146	1.000	0.422 *
Zn	0.615 *	0.172	0.332	0.330	−0.293	0.131	0.450 *	0.540 *	0.161	0.422 *	1.000

#### 4. Discussion

The cracking of fruit represents a significant economic loss and it will increase in the coming years due to the increase in rainfall and winds caused by climate change [10]. This work tested the induction of cracking through the use of different irrigation regimes, accompanied by foliar treatments to prevent cracking. Melon cracking has been associated with rapid water uptake that induces high cell turgor pressures, leading to rind rupture [28]. Therefore, both water and nutrient uptake must play a crucial role in cracking incidence and prevention [17].

The controlled induction of cracking is a useful tool for the study of cracking [10]. In this work, conductivity irrigation and double irrigation produced a significant increase in the cracking incidence on the day following their application, cracking being greater with the latter treatment. This is an

important point since the natural induction of cracking is related to unexpected and abundant rains in the summer [29], while the induction is less marked for irrigation with highly saline water [30], which is followed by normal irrigation. Therefore, since the induction of cracking is clearly due to a quick uptake of water, the amelioration should be related to the ability to control the increase in turgor. In our experiment, the fact that the irrigation treatments reduced the concentrations of most elements (with the exception of Na for conductivity irrigation), mainly in pulp, indicates a close correlation between resistance to cracking, which is the correct cellular composition of elements and water relations.

The alterations produced in our plants by the irrigation treatments reflect the fact that plants use stomata to maintain their transpiration and photosynthesis rates in the face of changes in the soil nutrient solution. In the conductivity irrigation shock treatment, the osmotic potential of roots, stems, and leaves should have increased. Therefore, when normal irrigation resumed, 12 h after the application of the irrigation treatments, the plants would have increased their water uptake, producing an increase in stomatal conductance and transpiration as a coordinated response [31]. However, the internal CO<sub>2</sub> concentration declined. This could be due to salt damage for the photosynthetic tissue, restricting the CO<sub>2</sub> availability for carboxylation [32]. The quick response of melon plants to salinity has been observed previously in terms of changes in gas exchange, indicating the good resiliency of this crop [33]. Double irrigation induced fewer changes in the gas exchange determinations in our melon plants. Waterlogging has been reported to limit plant growth directly, particularly by reducing the plant nutrient availability [34], but, in the short term, no severe changes are observed in gas exchange determinations or water relations [35]. Therefore, the increased cracking observed in these plants should be related to changes in water uptake/transport together with alterations in nutrient uptake. This explains why foliar treatments with nutrients are able to prevent cracking.

Previous studies used foliar nutrition with different mineral elements—mainly B, Ca, Mg, K, or Zn, among others—and the application of all these elements reduced the cracking in different fruits [17,19,25,26]. In our experiment, we applied Ca alone or combined with the rest of the elements, as well as a separate nutrient treatment without Ca. The Ca-based foliar treatments (Ca and Ca+m, with high Zn and B and low Mo), at the doses applied, did not decrease cracking, even though such treatments have been shown to decrease cracking in other species. This may have been because not enough Ca entered the plant, and, therefore, the fruit, to produce an effect since no significant differences in Ca were found in the leaves or in the fruit. Despite this, the microelement-based foliar treatment, m (B, Cu, Fe, Mn, Mo, and Zn), decreased the incidence of cracking significantly, which was mainly caused by double irrigation.

Despite the few differences between the foliar treatments, we found that there are some elements, mainly due to the type of irrigation, which increase their concentration in control irrigation, and the irrigation with less incidence of cracking, while others decrease in this. Control irrigation gave the highest concentrations of Zn in the rind and of B, Ca, Mg, Mn, P, and Zn in the pulp. Elements directly associated with the melon cracking zone were also found at higher levels, mainly in the rind. In the cracked zone (c), Mg, Mn, and P were increased and Na decreased with respect to the other two zones.

B is a very important microelement in the biosynthesis of cell walls and the development of new tissues [36]. It has been associated with the apiosyl residue of rhamnogalacturonan-II (RGII) with two monomers cross-linked by borate [37,38], providing cell wall elasticity [17]. In addition to its structural function, B also affects the permeability and integrity of membranes, increasing K permeability and the levels of Ca bound to membranes [39]. For B, we found moderate correlation with Ca (0.749 and 0.510) and low correlation with K (0.474 and 0.023) in the pulp and rind, respectively. These relationships are very clear, especially for the rind in the conductivity irrigation treatment, which gave an increase in Na while decreasing K uptake.

Ca application confers resistance and hardness to the fruit rind, and Ca participates in numerous processes as a molecular signalling agent, through its release into the cytoplasm. In addition to this, it is closely linked to the water balance processes of cells, being an osmolyte widely used by plants [40]. Ca strengthens the structure of the cell wall, modifying the activities of enzymes such as  $\beta$ -galactosidase,

$\beta$ -xylosidase, pectinmethylesterase, polygalacturonase or pectate lyase [41]. Finally, Ca is important in tissues such as the skin of the fruit, to which it gives resistance to different stresses [42]. For instance, it increases cuticle and epidermal thickness to protect the fruit from excessive water uptake [15]. This correlates with the increase in Ca in the pulp of the fruits of our plants receiving control irrigation, which had lower levels of cracking.

Mg is a key element in photosynthesis and in protein production [43]. It has many relationships with other elements. Some studies show that Mg and K compete to bind to ribosomes [44], while certain elements decrease Mg transport—such as K, Ca, or Mn [45]. In this sense, we found some strong and moderate correlations in pulp between Mg and Ca (0.832), K (0.673), and Mn (0.710), and, in rind, with Ca (0.568) and Mn (0.809) but not with K (0.131), pointing to the low transport of these elements in the rind. Furthermore, Mg has a structural involvement in cell walls since it is an important cofactor of numerous enzymes such as xylose isomerase, isocitrate lyase, or glutamine synthetase [46]. Hence, wall formation worsens under Mg deficiency.

Mn has structural functions since it affects processes such as lignin biosynthesis and amino acid synthesis [47]. Therefore, Mn affects the lignin levels of the fruit rind, reducing its hardness and resistance and increasing the incidence of cracking when deficient or making it too rigid and inflexible if present in excess. In our experiment, the increase in Mn in the pulp in the control irrigation treatment may have helped to maintain the integrity of the fruit.

Some forms of P, such as phytic acid, have a high affinity for elements like Ca, Mg, or Mn [48,49], as we can see with the moderate or strong correlation of P with these elements: 0.752, 0.619, and 0.803, respectively, for pulp and 0.558, 0.686, and 0.881, respectively, for rind. Therefore, the function of P in our system is linked to other elements, rather than a direct involvement in cracking.

Zn is necessary to maintain the integrity of membranes, their phospholipid levels, and the correct functioning of ion transport systems [50,51], thus affecting the capacity for water uptake and preventing the racking. In our plants, the Zn levels in both the pulp and rind were higher in the control than in the other two irrigation treatments, which could have influenced both water uptake and cracking.

Our results indicate that the transport of B, Ca, Mg, Mn, P, and Zn from leaves to fruit in melon plants and its relationship with lignin deserve more attention. In fact, the levels of expression of genes involved in lignin biosynthesis have been demonstrated to be related to cracking [52,53]. Additionally, the uptake of water/nutrients in relation to the dual transport of solutes and water by aquaporins [21] should be taken into account. The regulation of water intake could determine the internal fruit volume and the final cracking incidence [1].

## 5. Conclusions

The main differences in the nutrient composition of the pulp were caused by the irrigation treatments while the main differences in the rind (increases in Mg, Mn, and P, and a decrease in Na) were associated with the apparition of cracked areas. Furthermore, the increase in the cracking incidence in both irrigation treatment was associated with decreases in B, Ca, Mg, Mn, P, and Zn in pulp, decreases in B and Zn in rind, and increases in K in rind. Therefore, in the event of occasional heavy rain, simulated here with the double irrigation treatment, one way to reduce the incidence of cracking is the foliar application of a solution containing B, Cu, Fe, Mn, Mo, and Zn. This would support the needs of the fruits, avoiding cracking by establishing a multiple equilibrium among micronutrients. Further investigation into the amelioration of the cracking caused by high salinity is needed, since many more nutrients seem to be involved.

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